GENLISA® Urea Assay

REF : KBCA2341

Ver 1.1

Biochemical Assay for the Quantitative Determination of Urea in plasma and urine samples

RUO	**For Research Use Only**	**REF**	**Catalog Number**
	**Store At**	**LOT**	**Batch Code**
	**Manufactured By**		**Biological Risk**
	**Expiry Date**		**Consult Operating Instructions**

REF KBCA2341 Σ 96 tests

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Introduction:

Urea, the final product of protein metabolism, is the primary component of non-protein nitrogen. Blood urea nitrogen (BUN), which is mainly produced by the liver, is normally excreted in urine. However, patients with renal failure, nephritis, or urinary tract obstruction may have elevated levels of BUN.

Intended Use:

GENLISA® Urea Assay kit is intended for quantitative determination using a spectrophotometer of Urea in plasma and urine samples.

Principle:

In the presence of urease, urea is hydrolyzed to produce ammonium ions and carbon dioxide. When ammonium is present in an alkaline solution, it can react with a chromogenic agent to form a blue product. The optical density (OD) measured at 640 nm is directly related to the concentration of urea and can be used for calculation.

Materials Provided:

1. Reagent I: Enzyme Stock Solution - 0.1 ml
2. Enzyme Stock Solution Diluent - 30 ml
3. Reagent II: Chromogenic Reagent - 100 ml
4. Reagent III: Sodium Hypochlorite Solution-100 ml
5. Reagent IV: BUN Standard (6.006 mg/vial) - 3 vials

Handling/Storage:

1. All reagents should be stored as indicated on the component label and keep away from the light
2. All the reagents should be used within 12 months from manufacturing date.
3. Before using, bring all components to room temperature (18-25ºC). Upon assay completion ensure all components of the kit are returned to appropriate storage conditions.

Health Hazard Warnings:

1. Reagents that contain preservatives may be harmful if ingested, inhaled or absorbed through the skin.
2. For Research Use Only.

Reagent preparation:

Reagent I Solution: Dilute the stock solution with diluent with a ratio of 3:1000.
100 mM BUN Stock Solution: Dissolve each vial of BUN Standard (Urea powder) with double distilled water to 1 ml
10 mM BUN Solution: Dilute the stock solution with DDW at the desired rate.
Note: Keep all the reagents at 4ºC.

Sample Requirement:

Plasma should be collected with an anticoagulant such as oxalate, heparin, or EDTA. Blood urea nitrogen (BUN) levels remain stable at room temperature for up to 24 hours and at 4-6ºC for at least 7 days.

Urine samples can be diluted by 10 to 50 times their initial volume before measurement, and additional dilution can be performed if the results exceed the measurement range.

Assay Procedure:

1. Make tubes for **Blank, Standard,** and **Sample** and add the reagents according to the below table.

Compositions (ml)	Blank	Standard	Sample
DDW	0.02	-	-
Reagent IV Solution	-	0.02	-
Sample	-	-	0.02
Reagent I Solution	0.25	0.25	0.25
Mix thoroughly and warm the mixture in a water bath at 37ºC for exactly 10 min			
Reagent II	1	1	1
Reagent III	1	1	1

2. Blank the OD values at 640 nm using distilled deionized water (DDW) and read the optical density (OD) value of each well / tube.

Calculation Formula:

a. Formula

$$\frac{BUN\ Conc.}{mM} = \frac{OD_{Sample} - OD_{Blank}}{OD_{Standard} - OD_{Blank}} \times \frac{C_{Standard}}{10mM} \times \frac{CoD}{Pretreatment}$$

CoD represents the coefficient of dilution in the pretreatment process.

b. Example-

20 ul of human serum was measured, resulting in OD values of the Blank, Standard, and Sample being 0.018, 0.250, and 0.158, respectively.

$$\frac{BUN\ Conc.}{mM} = \frac{OD_{Sample} - OD_{Blank}}{OD_{Standard} - OD_{Blank}} \times \frac{C_{Standard}}{10mM} \times \frac{CoD}{Pretreatment} = \frac{0.158 - 0.018}{0.250 - 0.018} \times 10 \times 1$$
$$= 6.034mM$$

c. Reference value

The concentration of blood urea nitrogen (BUN) in human serum typically ranges from 2.9 to 8.2 mM.

Procedural Notes:

1. Dilution can be performed when the results are too high.
2. It is recommended to use disposable tubes to prevent contamination.
3. The Reagent I solution must be prepared before measurement due to its instability at room temperature.
4. The enzyme stock solution is viscous, so the addition should be minimal to avoid clinging to the tips.
5. The solution should be warmed for 10 minutes after the addition of reagent I. If the number of samples is too high, measurements should be done in batches of no more than 15 samples each.
6. The color produced remains stable for up to 4 hours.
7. The quality control can be performed using known serum samples available from the institute.

Safety Precautions:

- **This kit is For Research Use only**. Follow the working instructions carefully.
- The expiration dates stated on the kit are to be observed. The same relates to the stability stated for reagents
- Do not use or mix reagents from different lots.
- Do not use reagents from other manufacturers.
- Avoid time shift during pipetting of reagents.

- All reagents should be kept in the original shipping container.
- Since the kit contains potentially hazardous materials, the following precautions should be observed
- Do not smoke, eat or drink while handling kit material
- Always use protective gloves
- Never pipette material by mouth
- Wipe up spills promptly, washing the affected surface thoroughly with a decontaminant.
- In any case GLP should be applied with all general and individual regulations to the use of this kit.

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